

Vascular Medicine

Functional Lecithin: Cholesterol Acyltransferase Is Not Required for Efficient Atheroprotection in Humans

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Background—Mutations in the *LCAT* gene cause lecithin:cholesterol acyltransferase (LCAT) deficiency, a very rare metabolic disorder with 2 hypoalphalipoproteinemia syndromes: classic familial LCAT deficiency (Online Mendelian Inheritance in Man No. 245900), characterized by complete lack of enzyme activity, and fish-eye disease (Online Mendelian Inheritance in Man No. 136120), with a partially defective enzyme. Theoretically, hypoalphalipoproteinemia cases with LCAT deficiency should be at increased cardiovascular risk because of high-density lipoprotein deficiency and defective reverse cholesterol transport.

Methods and Results—The extent of preclinical atherosclerosis was assessed in 40 carriers of *LCAT* gene mutations from 13 Italian families and 80 healthy controls by measuring carotid intima-media thickness (IMT). The average and maximum IMT values in the carriers were 0.07 and 0.21 mm smaller than in controls ($P=0.0003$ and $P=0.0027$), respectively. Moreover, the inheritance of a mutated LCAT genotype had a remarkable gene-dose-dependent effect in reducing carotid IMT ($P=0.0003$ for average IMT; $P=0.001$ for maximum IMT). Finally, no significant difference in carotid IMT was found between carriers of *LCAT* gene mutations that cause total or partial LCAT deficiency (ie, familial LCAT deficiency or fish-eye disease).

Conclusions—Genetically determined low LCAT activity in Italian families is not associated with enhanced preclinical atherosclerosis despite low high-density lipoprotein cholesterol levels. This finding challenges the notion that LCAT is required for effective atheroprotection and suggests that elevating LCAT expression or activity is not a promising therapeutic strategy to reduce cardiovascular risk. (*Circulation*. 2009;120:628-635.)

Key Words: atherosclerosis ■ genetics ■ imaging ■ lipoproteins

A number of epidemiological studies have consistently demonstrated that a low plasma concentration of high-density lipoprotein cholesterol (HDL-C) is an independent risk factor for atherosclerotic vascular disease. Clinical sequelae are generally preceded by silent changes in the arterial wall, characterized by enhanced lipid deposition and infiltration of blood cells into the subendothelial space, with thickening of the arterial wall before formation of atherosclerotic plaques. HDL is believed to retard the formation of atherosclerotic lesions by removing excess cholesterol from cells¹ and by preventing endothelial dysfunction.² These mecha-

nisms may imply an increased thickening of the arterial wall and an enhanced formation of atherosclerotic plaques in individuals with low plasma HDL-C levels.

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Carotid intima-media thickness (IMT) accurately depicts the distance between blood-intima and media-adventitia interfaces of the carotid wall and is one of the best-established and most commonly used surrogate markers of atherosclerosis.³ It correlates with many cardiovascular risk factors,

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including low plasma HDL-C levels,^{4,5} and with the severity of coronary artery disease (CAD).⁶ More importantly, in prospective studies, carotid IMT predicts clinical CAD.⁷

A variety of molecular defects underlie primary hypoalphalipoproteinemia (Online Mendelian Inheritance in Man No. 604091), a group of rare inherited metabolic disorders, all characterized by extreme reductions of plasma HDL-C levels. Although the affected subjects should be exposed to accelerated atherosclerosis and enhanced CAD risk, these are not invariable findings. Paradigmatic is the case of rare mutations in the *APOA-I* gene causing hypoalphalipoproteinemia, some of which lead to enhanced carotid IMT and premature CAD,⁸ whereas others are associated with perfectly normal carotid IMT and CAD protection.⁹

Lecithin:cholesterol acyltransferase (LCAT) is a HDL-associated enzyme responsible for esterifying free cholesterol to cholesteryl ester within the plasma compartment.¹⁰ Homozygous mutations in the *LCAT* gene cause LCAT deficiency, a very rare metabolic disorder with 2 hypoalphalipoproteinemia syndromes: classic familial LCAT deficiency (FLD) (Online Mendelian Inheritance in Man No. 245900), characterized by complete lack of enzyme activity, and fish-eye disease (FED) (Online Mendelian Inheritance in Man No. 136120), with a partially defective enzyme.¹¹ Theoretically, hypoalphalipoproteinemia cases with LCAT deficiency should be at increased CAD risk because of HDL deficiency and defective reverse cholesterol transport. A review of scattered reports published until 1997 indicated that FLD cases generally do not present with premature CAD, which was instead described in a consistent number of FED cases.¹² However, the essentially anecdotal nature of CAD prevalence data in LCAT-deficient families leaves the issue of atherosclerosis and CAD risk in hypoalphalipoproteinemia with LCAT deficiency still open. We have recently identified 13 Italian families carrying 17 different *LCAT* gene mutations, the largest series of LCAT-deficient families reported to date.¹³ Here we report on the extent of preclinical atherosclerosis, as assessed by carotid IMT, in a large group of homozygous and heterozygous carriers of *LCAT* gene mutations from these Italian families.

Methods

Subjects

All members of the 13 previously identified LCAT-deficient families¹³ who were older than 18 years were asked to take part in the present study. Forty-six of the 82 eligible subjects volunteered for the study; these included 12 carriers of 2 mutant *LCAT* alleles, 28 carriers of 1 mutant *LCAT* allele, and 6 unaffected subjects. Because of the small number of available unaffected family members, a group of control subjects was recruited among blood donors (BD) attending the Servizio Immunoematologico Trasfusionale of the Niguarda Hospital in Milano.⁹ The BD database includes ≈7000 subjects; 276 of them could be matched for sex and age (up to 5 years older or younger than the carriers) to 1 of the carriers of mutant *LCAT* alleles. They were all asked to take part in the study if they did not have a personal history of cardiovascular disease; the first 80 subjects corresponding to each match who agreed to participate were selected for comparison (BD controls).

Medical personal and family history of cardiovascular diseases, presence of cardiovascular risk factors (smoking, hypertension,

diabetes, obesity), and use of medication were surveyed by questionnaires. Blood pressure, height, and weight were measured. Hypertension was defined as a blood pressure >140/90 mm Hg or use of antihypertensive medication. None of the participating subjects was taking drugs known to affect plasma lipid/lipoprotein levels. All subjects were fully informed of the modalities and end points of the study and signed an informed consent, and the procedures were approved by the institutional review board.

Carotid Ultrasonography

The 13 LCAT-deficient families are spread all around Italy. All carriers who agreed to participate in the study were asked to attend the Lipid Clinic of the Center E. Grossi Paoletti in Milano for IMT assessment. Twenty-two subjects traveled to Milano. All BD controls attended the same Lipid Clinic. In all of these subjects, ultrasound scanning of carotid arteries was performed by a single sonographer, with the use of an ESAOTE TECHNOS machine, equipped with a multifrequency probe of 8.5 MHz. When carriers could not travel to Milano, the sonographer moved to the family's site. In this case, ultrasound scanning was performed by using a portable device (Logiq E compact ultrasound portable system; GE), equipped with a 5.0- to 13.0-MHz linear array transducer. The same standard protocol, with scanning of the far walls of the right and left common carotid artery, bifurcation, and internal carotid artery in 3 different projections (anterior, lateral, and posterior), was used with both instruments.¹⁴ Videotape recordings were subsequently examined by a single expert reader using a specific software (M'Ath, Metris SRL France) that allows semiautomatic edge detection of the echogenic lines of the intima-media complex. Both sonographer and reader were blinded to the subject's identity. The far wall of the common carotid artery (in its entire length), the bifurcation, and the first proximal centimeter of the internal carotid artery were measured in at least 3 different frames. All measurements were averaged to calculate the average IMT for each subject; the highest IMT value among the 3 segments was defined as the maximum IMT. When IMT measurements obtained with the 2 ultrasound devices were compared by assessing carotid IMT in 20 subjects with varying degrees of preclinical atherosclerosis attending the Lipid Clinic,¹⁵ little bias was observed between the 2 devices (common carotid IMT: mean difference [MD]=−0.031 mm, limits of agreement [LOA]=−0.063, 0.001 mm; bifurcation IMT: MD=−0.013 mm, LOA=−0.089, 0.063 mm; internal carotid IMT: MD=−0.012 mm, LOA=−0.060, 0.037 mm; average IMT: MD=−0.017 mm, LOA=−0.036, 0.002 mm; maximum IMT: MD=−0.012 mm, LOA=−0.118, 0.094 mm).

Biochemical Analyses

Fasting blood samples were collected after an overnight fast. Plasma total cholesterol, HDL-C, triglyceride, and apolipoprotein levels were determined with certified methods by using a Roche diagnostics Integra 400 autoanalyzer. Plasma lipoproteins were separated by sequential ultracentrifugation with the use of a Beckman TL 100 ultracentrifuge equipped with a TL 100.3 rotor, and the cholesterol content of lipoprotein fractions was measured by enzymatic techniques.

Differential Diagnosis

Differential FLD and FED diagnosis in carriers of 2 mutant *LCAT* alleles was based on biochemical criteria.¹³ Because carriers of 1 mutant *LCAT* allele cannot be classified as FLD or FED on the basis of either biochemical or clinical criteria,¹¹ differential genetic diagnosis was performed by expressing LCAT mutants, as well as the wild-type LCAT protein, in COS-1 cells and measuring LCAT concentration and activity in the cell medium.

LCAT cDNA was cloned into the pSVNLCAT vector with the use of a double *Bam*HI site. This plasmid was used as a template to introduce the mutations by the QuikChange XL mutagenesis kit (Stratagene). Clones were propagated in XL1Blue bacteria cells and controlled by direct sequencing. COS-1 cells were grown in high-

Table 1. Demographic, Clinical, and Lipid/Lipoprotein Data in Carriers of *LCAT* Gene Mutations and BD Controls

	Carriers	Carriers of 2 Mutant <i>LCAT</i> Alleles	Carriers of 1 Mutant <i>LCAT</i> Allele	BD Controls	<i>P</i> (Carriers vs BD Controls)
n	40	12	28	80	
Sex, M/F	25/15	10/2	15/13	50/30	...
Age, y	41.7±17.0	32.4±5.9	45.7±18.7	41.5±16.7	...
BMI, kg/m ²	24.8±4.3	23.7±3.7	25.3±4.6	23.7±3.5	0.15
Smoking status, n (%)					0.78
Never	28 (70.0)	7 (58.3)	21 (75.0)	53 (66.0)	
Former	7 (17.5)	4 (33.3)	3 (10.7)	13 (16.3)	
Current	5 (12.5)	1 (8.3)	4 (14.3)	14 (17.5)	
Hypertension, n (%)	9 (22.5)	4 (33.3)	5 (17.9)	4 (5.0)	0.01
Total cholesterol, mg/dL	164.2±56.5	155.3±79.0	167.9±44.9	183.9±27.7	0.04
LDL-C, mg/dL	102.7±51.5	99.4±70.7	104.2±42.3	107.3±25.8	0.60
HDL-C, mg/dL	30.1±17.4	9.1±4.7	39.1±12.2	58.4±12.2	<0.0001
ApoA-I, mg/dL	83.3±34.2	39.5±8.3	102.1±21.1	140.6±22.9	<0.0001
ApoA-II, mg/dL	23.9±11.8	8.2±4.5	30.7±6.0	35.1±5.1	<0.0001
Triglycerides, mg/dL	156.5±111.3	234.5±165.4	123.0±53.7	95.6±66.5	0.002
Glucose, mg/dL	90.4±37.4	77.6±8.7	95.0±42.7	88.0±11.3	0.62

Data are reported as mean±SD unless indicated otherwise.

glucose Dulbecco's modified Eagle's medium media supplemented with 5% fetal bovine serum, 2 mmol/L L-glutamine, 100 UI/mL penicillin, 100 µg/mL streptomycin, 1 mmol/L Na-pyruvate, and ×1 nonessential amino acids. Cells were seeded into 60-mm dishes at a density of 400 000 cells per dish. Cells were transfected at 60% to 90% of confluence (24 hours after seeding) with Lipofectamine Reagent (Invitrogen, Carlsbad, Calif) or Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. The pSVD-NALCAT wild-type and mutant vectors were always cotransfected with pCMV β-Gal vector, expressing β-Gal gene, to assess the transfection efficiency. Twenty-four hours after transfection, the medium of transfected cells was substituted with a cell-free medium (Optimem, GIBCO) to avoid interference with the *LCAT* enzyme present in serum-containing medium. Seventy-two hours after transfection, the medium of transfected cells was collected and centrifuged for 10 minutes at 2000g at 4°C to remove cellular debris. Aliquots of the media were kept at −80°C until *LCAT* assays were performed. Plasma *LCAT* concentration was measured by an immunoassay.¹⁶ *LCAT* activity was measured with the use of reconstituted HDL and plasma-derived low-density lipoprotein (LDL) as substrate.¹³

Statistical Analyses

Data with near-normal distributions are presented as mean and SD. IMT variables, which have skewed distributions, were log-transformed before analysis and are presented as geometric means and 95% confidence intervals (CIs). Unadjusted comparisons of demographic and clinical data were performed by 2-sample *t* test. Unadjusted IMT values were compared by the Wilcoxon rank sum test. Comparisons adjusted for potential confounders (age, sex, body mass index [BMI], smoking status, hypertension, family history of cardiovascular disease, ultrasound device, and total cholesterol, HDL-C, and triglyceride levels) were performed by ANCOVA. Two models were tested to assess the effect of *LCAT* genotype on IMT measurements: a dominant model, with *LCAT* deficiency entered as a dichotomous variable, and a gene-dose model including the number of mutant *LCAT* alleles (0, 1, or 2). All tests were 2-sided, and *P* values <0.05 were considered statistically significant. All analyses were performed by SAS statistical package version 9.13 (SAS Institute Inc, Cary, NC).

Results

Carotid IMT in Carriers and Controls

A total of 120 subjects, 40 carriers of *LCAT* gene mutations (carriers) and 80 matched BD controls, participated in the study (Table 1). As per protocol, age was comparable in the 2 study groups; BMI, blood glucose, and smoking status were also similar. Three carriers and 8 controls had prediabetes, and 1 carrier had type 2 diabetes mellitus. Because of the inclusion of carriers of 2 mutant *LCAT* alleles, who frequently develop kidney disease,¹³ the prevalence of hypertension was greater in the carriers group (Table 1). As expected, carriers displayed marked reductions in HDL-C (Table 1), contributing to lower plasma total cholesterol levels than BD controls; there was no difference in LDL cholesterol (LDL-C) levels between the 2 groups. Carriers also had a greater fasting triglyceride level and lower apolipoprotein (apo)A-I and apoA-II levels than BD controls (Table 1). An evaluation of the LDL-C/HDL-C ratio, which is frequently associated with changes in cardiovascular risk,¹⁷ showed that this ratio was remarkably greater among carriers than among BD controls: 3.30 (2.07, 4.88) versus 1.82 (1.43, 2.44) (*P*<0.0001).

Mean carotid IMT values in the 2 study groups are reported in Table 2. The thickness of all 3 examined carotid segments was significantly lower in carriers than in BD controls. The difference in average IMT, which averages all IMT measurements in each individual, was highly statistically significant. The maximum IMT value, which reflects the presence of focal atherosclerotic lesions, was also significantly lower in carriers than in BD controls. After adjustment for age, sex, BMI, smoking status, hypertension, family history of cardiovascular disease, ultrasound device, and total cholesterol, HDL-C, and triglyceride levels, the difference in both average IMT and maximum IMT remained statistically significant

Table 2. IMT of the Common, Bifurcation, and Internal Carotid Artery Segments in Carriers of *LCAT* Gene Mutations and BD Controls

	Carriers	Carriers of 2 Mutant <i>LCAT</i> Alleles	Carriers of 1 Mutant <i>LCAT</i> Allele	BD Controls	<i>P</i> (Carriers vs BD Controls)	<i>P</i> (Trend)*
Unadjusted model						
Common carotid, mm	0.55 (0.52–0.59)	0.49 (0.44–0.55)	0.57 (0.54–0.62)	0.59 (0.56–0.61)	0.021	0.009
Bifurcation, mm	0.62 (0.56–0.69)	0.49 (0.41–0.59)	0.68 (0.61–0.77)	0.75 (0.70–0.80)	0.001	<0.0001
Internal carotid, mm	0.56 (0.52–0.60)	0.49 (0.42–0.56)	0.59 (0.54–0.65)	0.64 (0.60–0.67)	0.001	0.001
Average IMT, mm	0.58 (0.54–0.62)	0.50 (0.44–0.56)	0.62 (0.57–0.67)	0.65 (0.62–0.68)	0.0003	0.0005
Maximum IMT, mm	0.91 (0.81–1.02)	0.72 (0.59–0.88)	1.00 (0.88–1.14)	1.12 (1.03–1.21)	0.0027	0.0002
Adjusted model						
Common carotid, mm	0.55 (0.52–0.58)	0.52 (0.47–0.59)	0.55 (0.52–0.58)	0.59 (0.57–0.61)	0.108	0.04
Bifurcation, mm	0.62 (0.57–0.67)	0.54 (0.45–0.64)	0.63 (0.58–0.68)	0.76 (0.72–0.80)	0.015	0.0001
Internal carotid, mm	0.55 (0.52–0.59)	0.52 (0.45–0.60)	0.56 (0.52–0.59)	0.64 (0.61–0.67)	0.009	0.002
Average IMT, mm	0.58 (0.55–0.61)	0.53 (0.47–0.60)	0.58 (0.55–0.61)	0.66 (0.63–0.68)	0.0011	0.0003
Maximum IMT, mm	0.92 (0.84–1.00)	0.82 (0.68–1.00)	0.93 (0.84–1.01)	1.12 (1.06–1.19)	0.007	0.001

Data are reported as geometric mean and 95% CI. In the adjusted model, IMT values were adjusted for age, sex, BMI, smoking status, hypertension, family history of cardiovascular disease, ultrasound device, and total cholesterol, HDL-C, and triglyceride levels.

**P* for linear trend over the number of mutant alleles.

(Table 2). Two carriers, both heterozygote women, had a stroke at the age of 68 and 70 years.

Carotid IMT in Carriers of 2 and 1 Mutant *LCAT* Alleles

In a second analysis, the effect of the number of copies of mutant alleles (gene-dose effect) on average IMT and maximum IMT was investigated by ANCOVA, with the number of mutant alleles (0, 1, or 2) as independent variable and testing for trend. The characteristics of the present cohort of carriers of 2 and 1 mutant alleles (Table 1) were close to those previously reported for the entire series of identified carriers.¹³ After adjustment for age, sex, BMI, smoking status, hypertension, family history of cardiovascular disease, ultrasound device, and total cholesterol, HDL-C, and triglyceride levels, a mutation in the *LCAT* gene had a significant gene-dose-dependent effect on all IMT measurements (Table 2 and Figure). The gene-dose model fit the IMT data better than the dominant model ($F=10.7$ versus 6.8 for average IMT and $F=7.8$ versus 6.9 for maximum IMT). When adjusted IMT values in carriers of 2 or 1 mutant *LCAT* alleles were compared with those of BD controls (Table 2), a highly significant difference was found in both average IMT ($P=0.0009$ and $P=0.002$ for carriers of 2 and 1 mutant alleles, respectively) and maximum IMT ($P=0.01$ and 0.004 for carriers of 2 and 1 mutant alleles, respectively).

Carotid IMT in FLD and FED

In a third analysis, a comparison was made between subjects phenotyped as FLD and FED. Differential FLD and FED diagnosis in carriers of 2 mutant *LCAT* alleles was based on biochemical criteria¹³: 9 of the 12 examined carriers were classified as FLD and 3 as FED. Because carriers of 1 mutant *LCAT* allele cannot be classified as FLD or FED on the basis of either biochemical or clinical criteria,¹¹ differential genetic diagnosis was performed by expressing *LCAT* mutants in COS-1 cells and measuring *LCAT* concentration and activity

in the cell medium (Table 3). Twenty-four heterozygotes carry either a truncated *LCAT* mutant ($n=5$) or an *LCAT* mutant displaying undetectable activity against both reconstituted HDL and LDL substrates ($n=19$) and were thus classified as FLD. Four heterozygotes carry an *LCAT* mutant

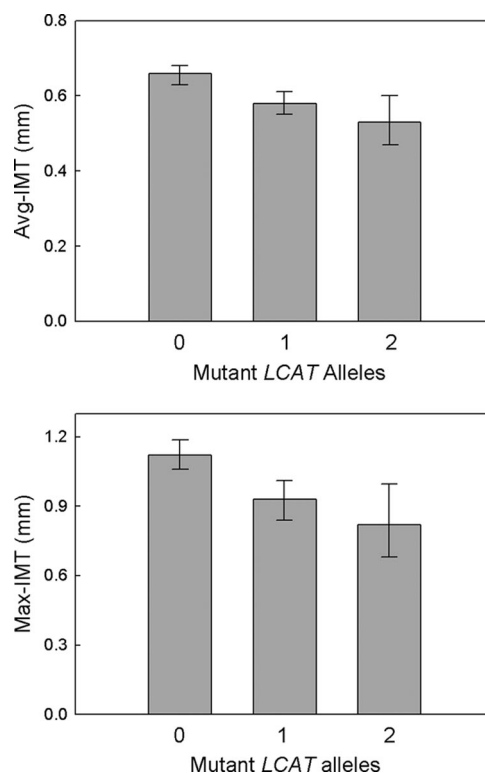


Figure. Average IMT (Avg-IMT) and maximum IMT (Max-IMT) values in carriers of 1 ($n=28$) or 2 ($n=12$) mutant *LCAT* alleles and in control subjects ($n=80$). IMT measurements were adjusted for age, sex, BMI, smoking status, hypertension, family history of cardiovascular disease, ultrasound device, and total cholesterol, HDL-C, and triglyceride levels. Data are expressed as geometric mean and 95% CIs. $P=0.0003$ for average IMT and $P=0.001$ for maximum IMT when tested for trend.

Table 3. Concentration and Activity of Missense LCAT Mutants Expressed in COS-1 Cells

Mutant	LCAT Mass, μg/mL	LCAT Activity, nmol/(mL·h)		Phenotype
		Reconstituted HDL	LDL	
WT	0.28±0.05	12.7±1.8	76.2±10.7	
S91P	0.40±0.14	0	0	FLD
A141T	0.27±0.08	0	117.3	FED
R147W	0.16±0.02	0	0	FLD
S181N	0.45±0.02	0	0	FLD
K218N	0.22±0.05	0	0	FLD
R244H	0.39±0.02	0	110.2	FED
T274I	0.27±0.06	0	0	FLD
V309M	0.32±0.03	0	0	FLD
L372R	0.16±0.07	0	0	FLD

Data are reported as mean±SD.

displaying no activity against reconstituted HDL but detectable activity against LDL and were classified as FED. IMT data from homozygous and heterozygous carriers with the same phenotype (either FLD or FED) were combined before analysis (Table 4). Average IMT and maximum IMT were similar in FLD and FED carriers and remained similar after adjustment for age, sex, BMI, smoking status, hypertension, family history of cardiovascular disease, ultrasound device, and total cholesterol, HDL-C, and triglyceride levels (Table

Table 4. Demographic, Clinical, Lipid/Lipoprotein, and IMT Data in FLD and FED Carriers

	FLD	FED	P
N	33	7	
Sex, M/F	20/13	5/2	0.69
Age, y	43.0±17.5	35.6±14.3	0.30
BMI, kg/m ²	24.8±4.6	25.2±2.4	0.86
Smoking status, n (%)			0.45
Never	22 (66.7)	6 (85.7)	
Former	7 (21.2)	0	
Current	4 (12.1)	1 (14.3)	
Hypertension, n (%)	8 (24.2)	1 (14.3)	0.71
Total cholesterol, mg/dL	164.1±52.4	164.6±78.1	0.98
LDL-C, mg/dL	99.3±47.6	119.1±69.0	0.36
HDL-C, mg/dL	31.8±17.7	22.0±14.3	0.18
ApoA-I, mg/dL	85.2±34.8	74.3±32.4	0.45
ApoA-II, mg/dL	23.7±11.7	25.1±13.5	0.76
Triglycerides, mg/dL	164.7±119.0	117.4±53.7	0.32
Glucose, mg/dL	91.3±39.1	80.3±8.1	0.63
Average IMT, mm	0.58 (0.53–0.64)	0.56 (0.46–0.69)	0.72
Maximum IMT, mm	0.93 (0.81–1.07)	0.81 (0.60–1.10)	0.42
Adjusted average IMT, mm	0.66 (0.57–0.78)	0.59 (0.54–0.64)	0.15
Adjusted maximum IMT, mm	0.94 (0.75–1.19)	0.97 (0.86–1.09)	0.84

Data are reported as mean±SD unless indicated otherwise. IMT data are expressed as geometric mean (95% CI). In the adjusted model, IMT values were adjusted for age, sex, BMI, smoking status, hypertension, family history of cardiovascular disease, ultrasound device, and total cholesterol, HDL-C, and triglyceride levels.

4). However, this nonsignificant result should be taken with caution because, given the observed variability, the limited sample size allowed us to detect as significant (with $\alpha=0.05$) an estimated difference of 0.19 mm between the 2 groups with an 80% statistical power.

Discussion

In the present study, we took advantage of the previous identification of 13 hypoalphalipoproteinemia families carrying different mutations in the *LCAT* gene¹³ to investigate the impact of defective LCAT function on preclinical atherosclerosis, as assessed by measuring carotid IMT. Unexpectedly, the low HDL-C levels in carriers of *LCAT* gene mutations do not result in premature atherosclerosis. Indeed, the inheritance of a mutated LCAT genotype has a remarkable gene-dose-dependent effect in reducing carotid IMT. Moreover, there is no difference in carotid IMT between carriers of *LCAT* gene mutations that cause total or partial LCAT deficiency (ie, FLD or FED).

Three different data analyses have been performed in the present study. First, we compared IMT data from carriers of *LCAT* gene mutations (1 and 2 mutated alleles together) with those from a double number of age/sex-matched healthy controls. On average, carriers had a 0.07 mm smaller average IMT and a 0.21 mm smaller maximum IMT than controls, a difference that remained statistically significant after adjustment for a variety of cardiovascular risk factors known to affect artery wall thickness. Mean average IMT and maximum IMT in carriers of *LCAT* gene mutations were 0.22 (95% CI, 0.06 to 0.37) mm and 0.52 (95% CI, 0.14 to 0.90) mm smaller than IMT measurements made by the same sonographers in 55 slightly older individuals with hypoalphalipoproteinemia, after adjustment for age.⁴ Notably, both IMT measurements were strikingly similar to those measured in individuals with hypoalphalipoproteinemia of approximately the same age carrying the atheroprotective apoA-I_{Milano} mutation.⁹ Therefore, LCAT deficiency is another monogenic disorder of HDL metabolism leading to a paradoxical phenotype characterized by moderate to severe HDL deficiency without enhanced preclinical atherosclerosis.

In a second analysis, all the examined subjects were subgrouped according to the number of mutant alleles, and a highly significant gene-dose-dependent effect of the LCAT genotype on carotid IMT was found. The inheritance of a single mutant *LCAT* allele leads to an extent of preclinical atherosclerosis intermediate between that of carriers of 2 or 0 copies of mutant alleles. A striking *LCAT* gene-dose-dependent effect on cholesterol esterification and other HDL-related biomarkers has been found in the same families,^{13,18} indicating that both the biochemical and the vascular changes are expressed as codominant traits in families carrying mutations in the *LCAT* gene.

The third analysis was aimed at comparing IMT values from carriers of *LCAT* gene mutations causing complete or partial (ie, FLD and FED) enzyme inactivation. On the basis of genotype, 7 carriers of 2 mutant alleles (all FLD) were homozygotes for the same mutation, and 5 (2 FLD and 3 FED) carried 2 distinct mutations.¹³ When mutations were categorized by site-directed mutagenesis, the 2 compound

heterozygotes with FLD were found to carry 2 different FLD mutations, whereas the 3 compound heterozygotes with FED were found to carry 1 FLD and 1 FED mutation. In the latter cases, the combined effect of the 2 mutations might have resulted in some kind of intermediate phenotype, whose clinical features were not easily distinguishable from those of classic FED. Obviously, any transposition of an *in vitro* biochemical property of the mutant (activity against LDL but not HDL) to the *in vivo* situation is an oversimplification in terms of the clinical phenotype found in the carriers. Because of the small number of carriers, data from homozygotes, compound heterozygotes, and heterozygotes with the same phenotype (either FLD or FED) were combined in this analysis. Both average IMT and maximum IMT were similar in FLD and FED carriers and remained similar after adjustment for a variety of cardiovascular risk factors. The investigation of the biochemical phenotype of these same carriers demonstrated that, despite the different functional defect of the LCAT enzyme, the lipid/lipoprotein phenotype of FLD and FED carriers is largely indistinguishable¹³; here we show that the same occurs at the vascular level. Altogether, these findings support the concept that FLD and FED are not 2 distinct syndromes but the same disease at different levels of LCAT impairment.

LCAT catalyzes the transacylation of the *sn*-2 fatty acid of lecithin to the free 3-OH group of cholesterol, generating cholesteryl ester and lysolecithin,¹⁹ and plays a central role in intravascular HDL metabolism and in reverse cholesterol transport,¹⁰ the process believed to explain HDL-mediated atheroprotection. A defect in LCAT function is thus expected to enhance atherosclerosis by interfering with these processes. The present data demonstrate exactly the opposite: In the subjects studied here, a partial or complete dysfunction of the LCAT enzyme is associated with reduced preclinical atherosclerosis. This unexpected finding merits some speculation. The inheritance of a defective LCAT enzyme may not preclude cholesterol removal from the arterial wall and efficient reverse cholesterol transport. Indeed, sera from these same carriers of *LCAT* gene mutations displayed an enhanced capacity for ABCA1-mediated cell cholesterol efflux than sera from unaffected family members because of the greater content of pre- β -HDL particles.¹⁸ Notably, LCAT-deficient mice display a preserved reverse cholesterol transport *in vivo* despite the severe HDL-C reduction, primarily because of the high plasma content of pre- β -HDL and enhanced macrophage cholesterol removal via ABCA1.²⁰ Moreover, kinetic studies in humans show that unesterified cholesterol within HDL may be efficiently delivered to the liver and excreted into bile without conversion into cholesteryl ester.²¹ Nevertheless, the preserved reverse cholesterol transport might not by itself justify the reduced preclinical atherosclerosis observed in the carriers, which may result from delayed cholesterol deposition in the arterial wall, as a consequence of a reduced cholesteryl ester content of apoB-containing lipoproteins,²² or a redistribution of HDL-bound antioxidant enzymes, like paraoxonase-1, or both to apoB-containing lipoproteins.²³

The effect of LCAT on human atherogenesis has been controversial. Early cross-sectional studies have reported both decreased^{24,25} and increased²⁶ LCAT activity in patients

with angiographically proven CAD. No prospective studies relating baseline LCAT activity/concentration to future cardiovascular events have been performed yet. Genetic LCAT deficiency may thus be a valuable model to investigate the relationship between LCAT and CAD, but the small number of events in the limited series of affected individuals investigated thus far and the essentially anecdotal nature of these reports are of little help in clarifying this issue. Carotid IMT has been established as a surrogate marker for human atherosclerosis³ and has been applied to the study of human atherosclerosis in hypoalphalipoproteinemia patients carrying mutations in the *apoA-I*, *ABCA1*, and *GBA* genes.^{8,9,27,28} Notably, despite the common hypoalphalipoproteinemia trait, carriers of different mutations display carotid IMT values that are greater than^{8,27} or close to^{9,28} those of controls. Three recent studies evaluated the extent of preclinical atherosclerosis in carriers of *LCAT* gene mutations by measuring carotid IMT. In the first study, 2 homozygotes and 7 heterozygotes, aged up to 70 years, from a large Canadian kindred with FLD have been followed for >25 years, during which none had suffered any vascular events.²⁹ No control (unaffected) subjects were included for comparison. Both homozygotes had a carotid IMT "slightly over the 75th percentile for their sex and age,"²⁹ which did not progress over 4 years (1997–2001); they also had normal endothelial function, as measured by flow-mediated dilation of brachial artery. The heterozygotes had greater carotid IMT than homozygotes; 4 of them had a carotid IMT >75th percentile. On average, the heterozygous carriers had a 0.236 mm greater total carotid IMT than the expected reference value.²⁹ In the second study, 9 homozygotes (or compound heterozygotes) and 47 heterozygotes from 5 Dutch FED families were investigated; 2 heterozygotes had premature CHD.³⁰ The homozygotes had greater average carotid IMT than controls, but the low number of cases and the relatively old age did not provide adequate power for statistical evaluation; the heterozygotes had slightly greater average carotid IMT than controls (0.032 mm).³⁰ The third study examined a limited number of American hypoalphalipoproteinemia patients, some of whom carry mutations in the *LCAT* gene; mean carotid IMT values were not different between hypoalphalipoproteinemia cases and controls.³¹ These previous findings are clearly at variance with those found in the present study. The explanation for this discrepancy is not readily apparent, although it may be related to a distinctly lower prevalence of cardiovascular risk factors other than low HDL-C in the Italian LCAT-deficient families. Indeed, slightly lower mean plasma LDL-C and BMI values were found in the Italian than other carriers. Moreover, in the Dutch study, a greater prevalence of male subjects and smokers among carriers than controls may have biased the results in favor of enhanced IMT among LCAT-deficient heterozygotes.³⁰

Some aspects of this study merit caution. The study sample was relatively small, despite the great effort in recruiting as many carriers as possible. However, the IMT studies in other rare genetic HDL disorders mentioned above have examined cohorts of the same size.^{9,29,30} A potential limitation in studies of small cohorts like this one is the referral basis of the examined subjects. Almost half of the Italian probands were

identified and referred to us by lipid clinics because of low HDL-C level, with the others being identified and referred by departments of nephrology because of impaired renal function. Both conditions have been independently associated with enhanced carotid IMT, thus excluding a bias for selecting probands carrying *LCAT* gene mutations associated with reduced preclinical atherosclerosis and cardiovascular disease risk. Another possible limitation of this study lies in the lack of unaffected family members as controls; however, only 6 unaffected relatives, with a sex-age distribution remarkably different from that of carriers, provided consent to ultrasound investigation.

In conclusion, the present study shows that genetically determined low-*LCAT* activity in Italian families is not associated with enhanced preclinical atherosclerosis despite low HDL-C levels. This finding challenges the notion that *LCAT* is required for effective atheroprotection and suggests that, despite positive effects on plasma HDL-C concentration, elevating *LCAT* expression or activity is not a promising therapeutic strategy to reduce cardiovascular risk.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Population studies clearly demonstrate that a low plasma high-density lipoprotein cholesterol (HDL-C) concentration is a strong predictor of future coronary heart disease. Genetically determined low HDL-C states, however, may be associated with a widely variable cardiovascular risk. In the present study, we show that individuals with a defect in cholesterol esterification, due to mutations in the gene coding for the lecithin:cholesterol acyltransferase enzyme, have low HDL-C levels but do not present with enhanced preclinical atherosclerosis, as assessed by measuring carotid intima-media thickness. This finding complements previous observations on the lack of preclinical atherosclerosis and premature coronary heart disease in other genetic conditions leading to reduced plasma HDL-C levels, like the apolipoprotein A-I_{Milano} mutation. It illustrates that HDL-C levels per se do not necessarily reflect the atheroprotective potential of HDL-C and highlights the need for novel tools for cardiovascular risk prediction in individuals with low HDL-C. Moreover, it challenges the notion that lecithin:cholesterol acyltransferase is required for effective atheroprotection and suggests that, despite positive effects on plasma HDL-C concentration, elevating lecithin:cholesterol acyltransferase expression or activity is not a promising therapeutic strategy to reduce cardiovascular risk.